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OCCURRENCE OF MACROINVERTEBRATES IN A PUBLIC DRINKING WATER SUPPLY

by

Richard V. Levy, Ronald D. Cheetham, and Frederick L. Hart
Worcester Polytechnic Institute
Worcester, MA 01609

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Eugene W. Rice

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
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16. ABSTRACT

This manuscript reports on the occurrence of amphipods in potable water. These organisms may be the cause of taste and odor problems as well as serving as potential carriers of harmful bacteria. Disinfection data was presented showing the higher resistance to chlorine by bacteria associated with the macroinvertebrates as opposed to the unassociated.

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Occurrence of Macroinvertebrates in a Public Drinking Water Supply

Richard V. Levy
Research Associate
Department of Biology and Biotechnology
Worcester Polytechnic Institute
Worcester, Massachusetts 01609

Ronald D. Cheetham
Associate Professor
Department of Biology and Biotechnology
Worcester Polytechnic Institute
Worcester, Massachusetts 01609

Frederick L. Hart
Associate Professor
Department of Civil Engineering
Worcester Polytechnic Institute
Worcester, Massachusetts 01609

A persistent problem associated with current drinking water resources is the appearance and persistence of micro- and macroinvertebrates in both filtered and unfiltered water supplies and distribution systems. These invertebrates include crustaceans, (amphipods, copepods, isopods, ostracods), nematodes, flatworms, water mites, and insect larvae such as chironomids. Their presence in potable water distribution systems may be anticipated, but should not be tolerated. These invertebrates may be the cause of tastes and odors, water discoloration, point-of-use filter and or water meter clogging, high turbidity, political embarrassment and public health concerns.

Macroinvertebrate colonization of distribution systems has been substantiated. We and others (1,2), suggest that selected macroinvertebrates live and reproduce within distribution systems, while others are simply sorted hydraulically throughout the pipe network. Recent studies on the nature of the encrustations and tubercules within pipelines (1,3,4) suggest that there are adequate food sources (direct or indirect) so that certain "benthic" macroinvertebrates may actually infest sections of a distribution system that provide a favorable habitat.

MacKenthun and Keup (5) stated that perhaps none of these macroinvertebrates found in waters used for domestic purposes are injurious to health. The perhaps is indeed significant for no reference was given to substantiate this claim. Gerardi and Grimm (6) expressed the opinion of the majority of water purveyors and researchers when they wrote that for lack of any epidemiological or microbiological data to the contrary "these animals are not known to present a serious public health risk."

The potential public health significance of macroinvertebrates was demonstrated to the satisfaction of many by Chang, Woodward, and Kabler (7) who reported that nematodes were capable of ingesting enteric bacterial pathogens and viruses. These pathogens were

afforded protection from chlorination. Tracy, Camarena, and Wing (8) theorized that microbiota such as coliforms become encapsulated within copepods and are afforded protection from chlorination. They further theorized that upon reaching the consumers tap, many of these microcrustaceans have broken apart or have disintegrated releasing the encapsulated microbiota. Haney (9) reported that macroinvertebrates (in this case nematodes) could pose a threat to the public health if they were exposed to potential pathogens. Most recently Huq et al. (10) suggested that the attachment of Vibrio cholerae to live copepods might be an important factor in the ecology of this species as well as in the epidemiology of cholera. We suggest that macroinvertebrates could provide a possible mode of transmission and protection for naturally associated or recently adsorbed bacteria and viruses.

Since contamination of potable waters may occur both from within: backsiphonage, cross-connections, leaks and repairs, deficient filtration and disinfection and from without: animal defecation and contamination, pasture run-off and improper waste disposal, macroinvertebrates could come into contact with microbiological contaminants at multiple locations.

To better understand micro and macroinvertebrate occurrences and to evaluate the public health significance of such events, we began to examine those organisms which are found in the distribution system of Worcester, Massachusetts.

METHODS

To test the hypothesis that micro and macroinvertebrate occurrences constitute a public health hazard, we developed techniques which :

I. Quantify and identify the normal flora of macroinvertebrates;

II. Test the adsorption of test bacteria to a model macroinvertebrate;

III. Evaluate the chlorine and chloramine disinfection kinetics of both natural and laboratory associated (seeded) bacteria.

At the same time we instituted a systematic sampling program to locate and identify these invertebrates in the distribution system of Worcester, Massachusetts.

A laboratory procedure was developed to quantify, culture, and identify the naturally occurring bacterial flora associated with macroinvertebrates derived from reservoirs and distribution systems (Figure 1). Hyalella azteca (Crustacea Amphipoda) was chosen as a model invertebrate. Specimens were collected by dip netting in shallow areas of reservoirs and from sites within the potable water distribution system from point-of-use filters or hydrants. Following sampling and washing in filter sterilized ambient water, amphipods were homogenized and the resultant homogenate plated onto both Plate Count Agar (PCA) and R-2A low nutrient agar. Bacteria were enumerated, described physically, gram stained, and

subcultured to the same agars on which they were originally isolated. Subcultures were subjected to a battery of biochemical tests designed to identify the isolates. Selected isolates were also tested with either the API 20E (Analytab Products, Plainville, N.Y.) or NFT (DMS Laboratories, Inc., Darts Mill, N. J.) identification systems. Concurrently macroinvertebrates were prepared for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) and examined both externally and most recently internally for bacterial colonization.

Water samples were taken at the same time from the reservoir habitat or from sites of macroinvertebrates' occurrences to quantify, culture, and identify bacteria using the above methodology. In addition to PCA and R-2A spread plates, Membrane Filtration and Most Probable Number Tests (11) were also run on water samples. These methods allow for the culturing of heterotrophic and indicator bacteria of the ambient water.

An experimental procedure was developed to study the association of selected bacteria and macroinvertebrates and their response to free available chlorine and monochloramine disinfection. This procedure incorporates the seeding (adsorption) of a selected, morphologically and biochemically distinct bacterium onto a macroinvertebrate and the subsequent disinfection of those newly adsorbed bacteria in a batch reactor system.

In addition to the results reported in this article, other studies in progress are evaluating the response of the natural bacterial flora of H. azteca to disinfection with chlorine and chloramines. The natural flora of copepods derived from the reservoirs and distribution systems are also being studied.

RESULTS

Data analyzed over a period of two years indicate that macroinvertebrates derived from both reservoirs and distribution systems are colonized by bacteria as evidenced by culturing, SEM and TEM examination.

Bacteria isolated from the macroinvertebrate H. azteca include species from the following genera:

- Actinomyces
- Aeromonas
- Bacillus
- Chromobacterium
- Klebsiella
- Pseudomonas
- Staphylococcus

The diversity of bacteria associated with amphipods is consistently lower than that of the bacteria associated with water samples taken concurrently. The most commonly isolated genus is Pseudomonas from both reservoir and distribution system specimens. Generally speaking, the bacteria isolated from amphipods from the distribution system are of the same species as those cultured from reservoir derived amphipods.

The culturing of amphipod associated flora (as measured by number of colony forming units (CFU) on spread plates of amphipod homogenates) indicate that colonization increases with age or residence time in the environment (Figure 2). Therefore, amphipods entering a water distribution system during fall might be expected to be supporting a larger population of bacteria than the same organisms entering in the winter or spring.

SEM examination of amphipod surfaces reveals that these bacteria are adsorbed onto the external exoskeleton, and form permanent associations. They are frequently seen as microcolonies surrounding the mouth (Figure 3) and anus, and inside joint cavities. More exposed external surfaces (e.g. the lateral and dorsal surfaces) are less often colonized. When external surfaces are colonized by bacteria the bacteria are firmly attached by extracellular polymeric substance (EPS).

SEM and TEM of amphipod gut tract analyses indicate that bacteria are associated with the gut tract. In specimens derived from the distribution system, the gut tracts are often colonized with cocci thought to be Staphylococcus (Figure 4).

To date seeding and disinfection studies have been completed under selected laboratory conditions using Escherichia coli (Lac Z545) and Enterobacter cloacae (ATCC 23355) and the amphipod Hyalella azteca at 20°C.

The magnitude of bacterial association was shown to be a function of macroinvertebrate size (we believe surface area to be most important); the larger the amphipod, the greater the adsorption (Figure 5). This observation led to the standardization of amphipod size in the experiments reported here. E. coli was shown to bind tightly (1.6×10^4 CFU/amphipod) while E. cloacae was shown to bind less tightly (less than 1.0×10^3 CFU/amphipod). E. cloacae was less resistant to removal by repeated washing than E. coli. Over longer periods of time (at least 5 days) numbers of associated E. cloacae increased initially then decreased to less than 100 CFU/amphipod. These bacteria appear to be firmly adsorbed and resist attempts to remove them.

In experiments comparing the adsorption of E. coli to both live and freshly killed amphipods, no significant difference in CFU adsorbed was noted.

At 20°C seeded bacteria are more resistant to disinfection with chlorine and chloramines than free (unassociated) bacteria. Unassociated E. coli populations decreased to less than 1% of their zero time controls within one minute of contact time, whereas more than 2% of associated E. coli remained viable after 60 minutes of contact time at 1.0 mg/l free available chlorine (Figure 6).

Unassociated E. cloacae populations decreased to less than 1% of their zero time controls within one minute of contact time, while approximately 15% of the associated E. cloacae remained viable after 60 minutes of contact time at 1.0 mg/l free available chlorine (Figure 7).

Monochloramine (at pH 8.0 and 20°C) has proved to be less effective at inactivating associated E. cloacae than free available chlorine at the same concentrations (Figure 8). Approximately 20% of the associated E. cloacae remained viable after 60 minutes contact time at 1.0 mg/l monochloramine. At all concentrations of monochloramine tested (0.5, 1.0 and 2.0 mg/l) the number of recoverable bacteria increased after 120 minutes contact. Inactivation experiments at 4°C are expected to yield significantly less efficient inactivation of macroinvertebrate associated bacteria.

CONCLUSIONS

From our current data we conclude that H. azteca does support a population of bacteria and that these bacteria are afforded protection from disinfection with free available chlorine and monochloramines under laboratory conditions. Bacteria recently adsorbed to Hyalella are also afforded protection from chlorination when compared to unassociated bacteria. Macroinvertebrates may constitute a health hazard if they become colonized with or are exposed to and adsorb potential bacterial pathogens.

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DISCLAIMER

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FLOW DIAGRAM FOR MICROBIOLOGICAL ANALYSIS OF WATER AND OF MACROINVERTEBRATES AND THEIR ASSOCIATED MICROBIOTA.

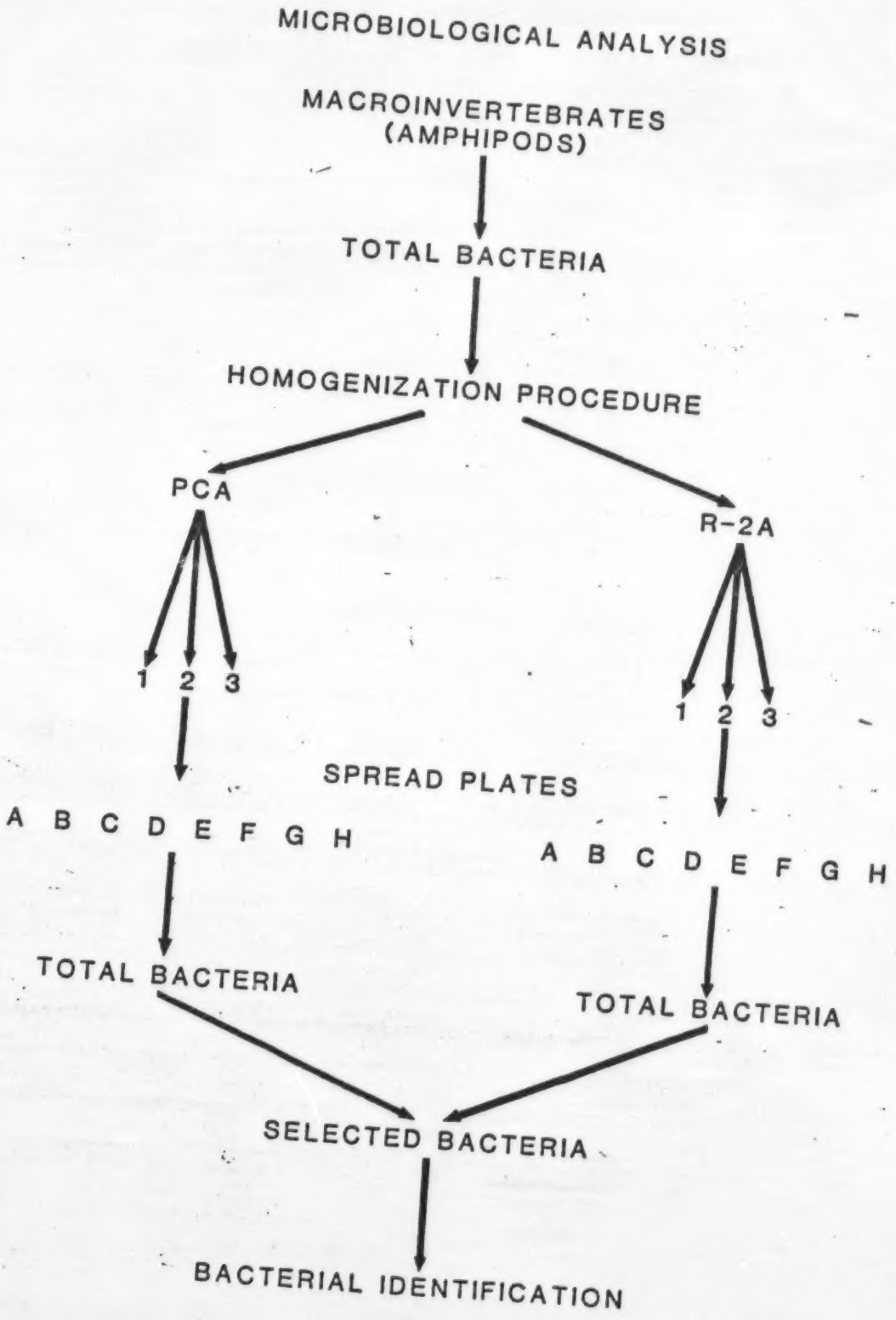
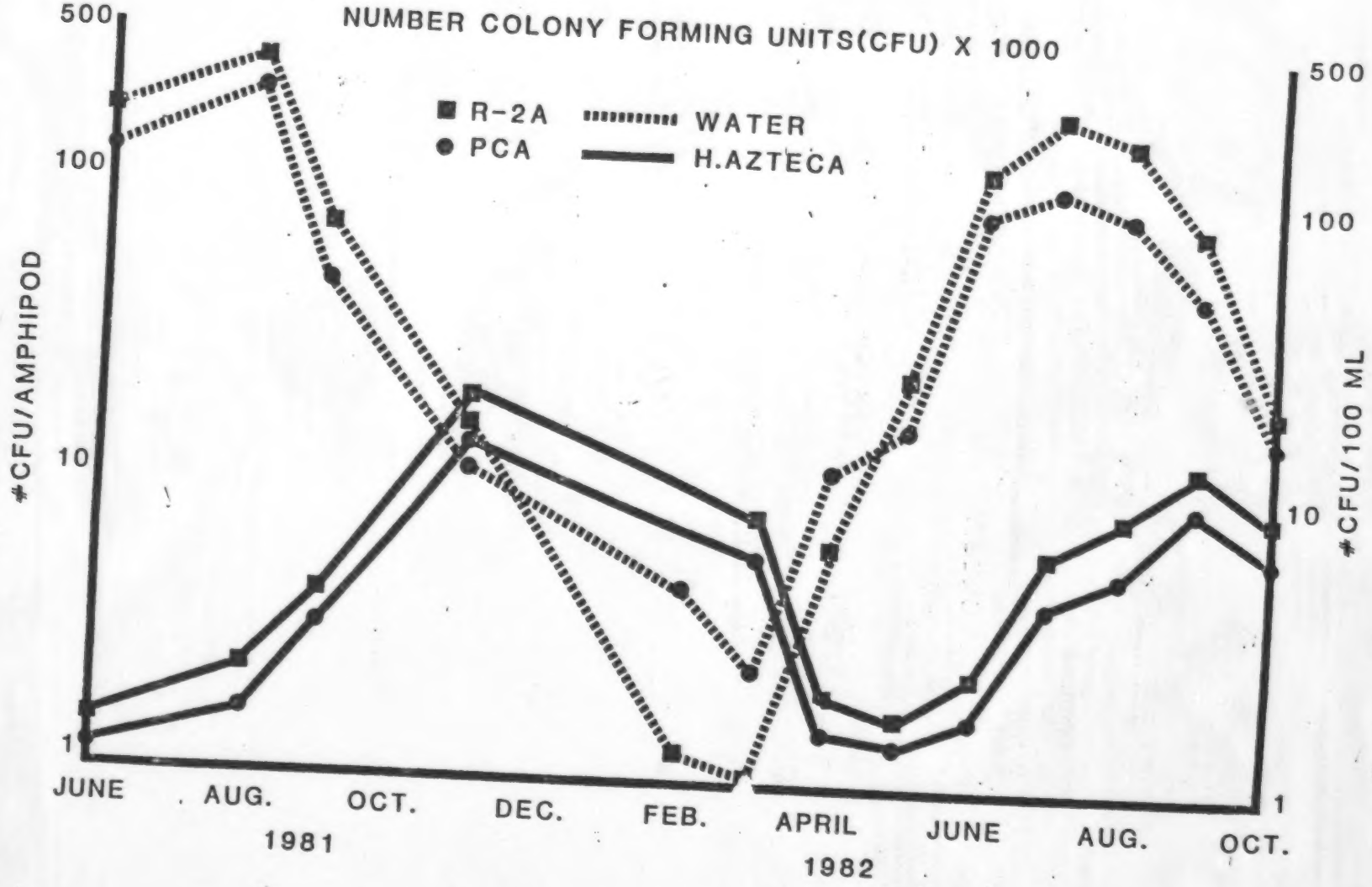
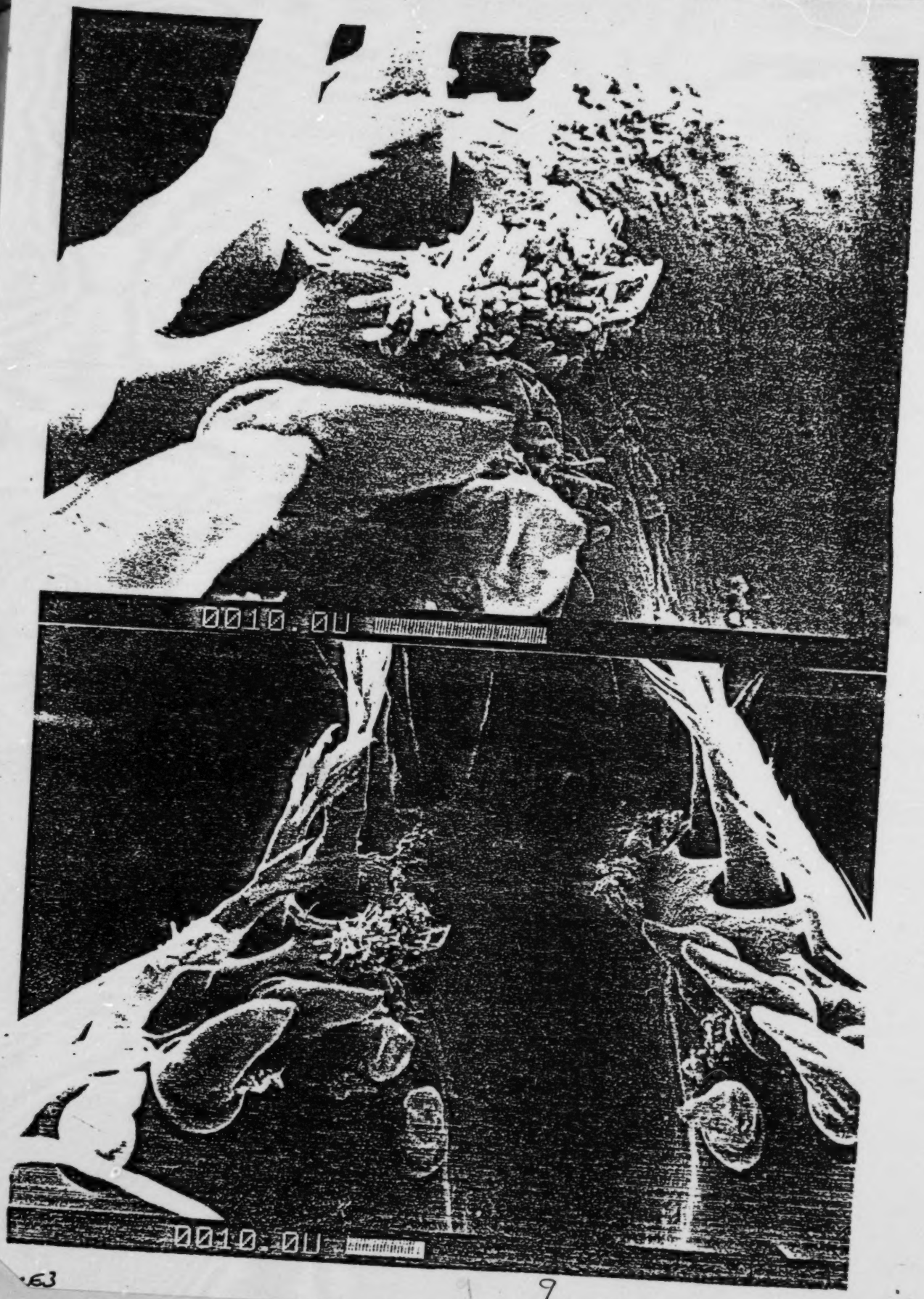


FIGURE 1

VARIATION IN NUMBER OF BACTERIA(CFU) ASSOCIATED WITH THE WATER COLUMN AND BACTERIA ASSOCIATED WITH MACROINVERTEBRATES FROM HOLDEN I AND II RESERVOIRS, WORCESTER, MASSACHUSETTS.

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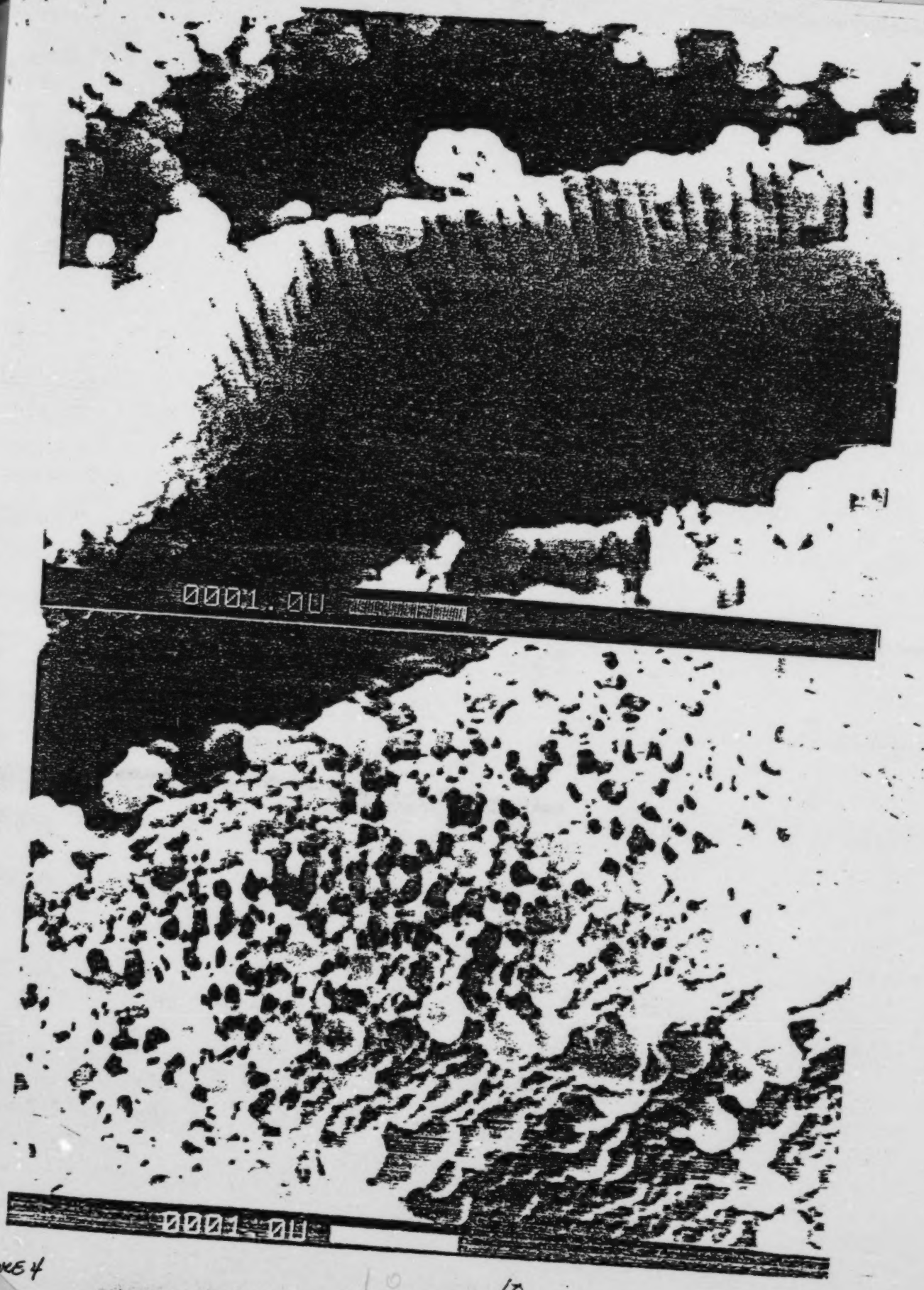
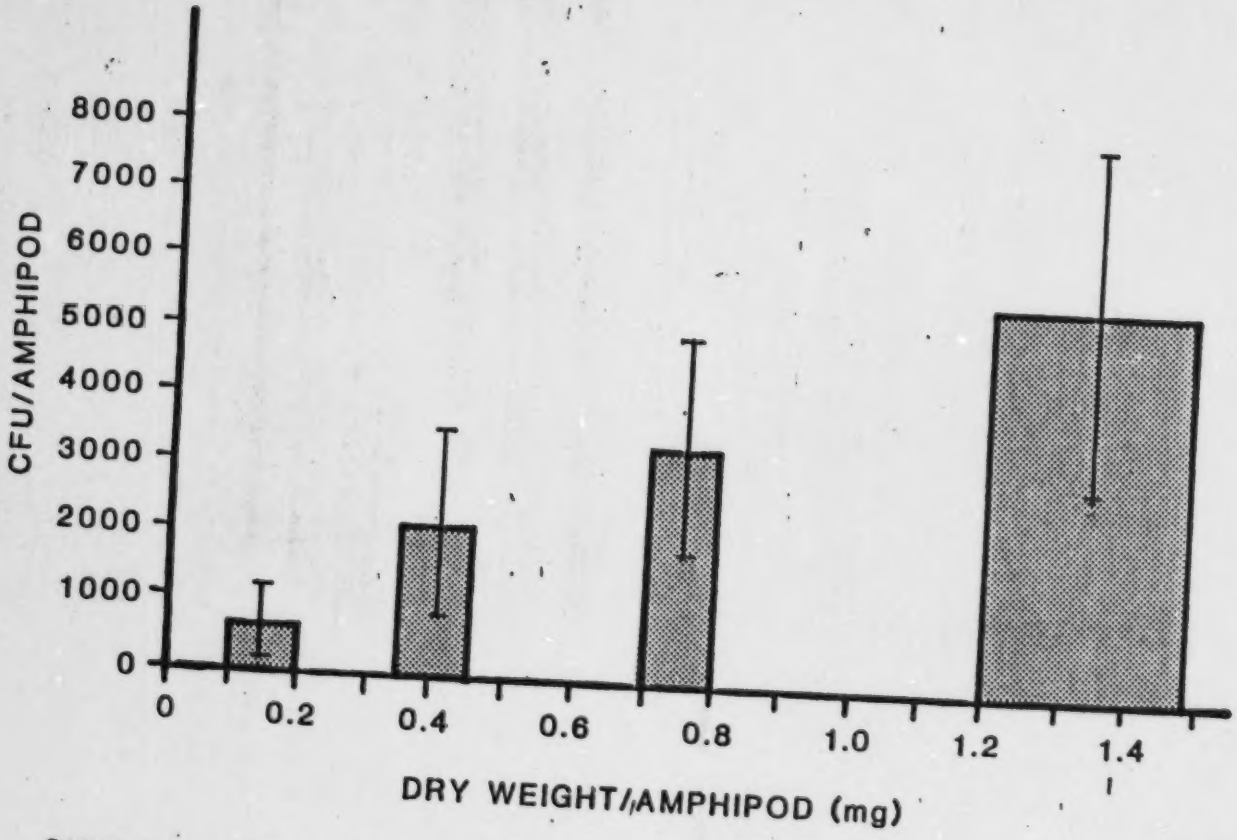


MKE 4



SIZE OF AMPHIPOD (AS MEASURED BY DRY WEIGHT) COMPARED TO NUMBER OF E.COLI ADSORBED AT 20°C AFTER 6 HOURS SEEDING

FIGURE 5

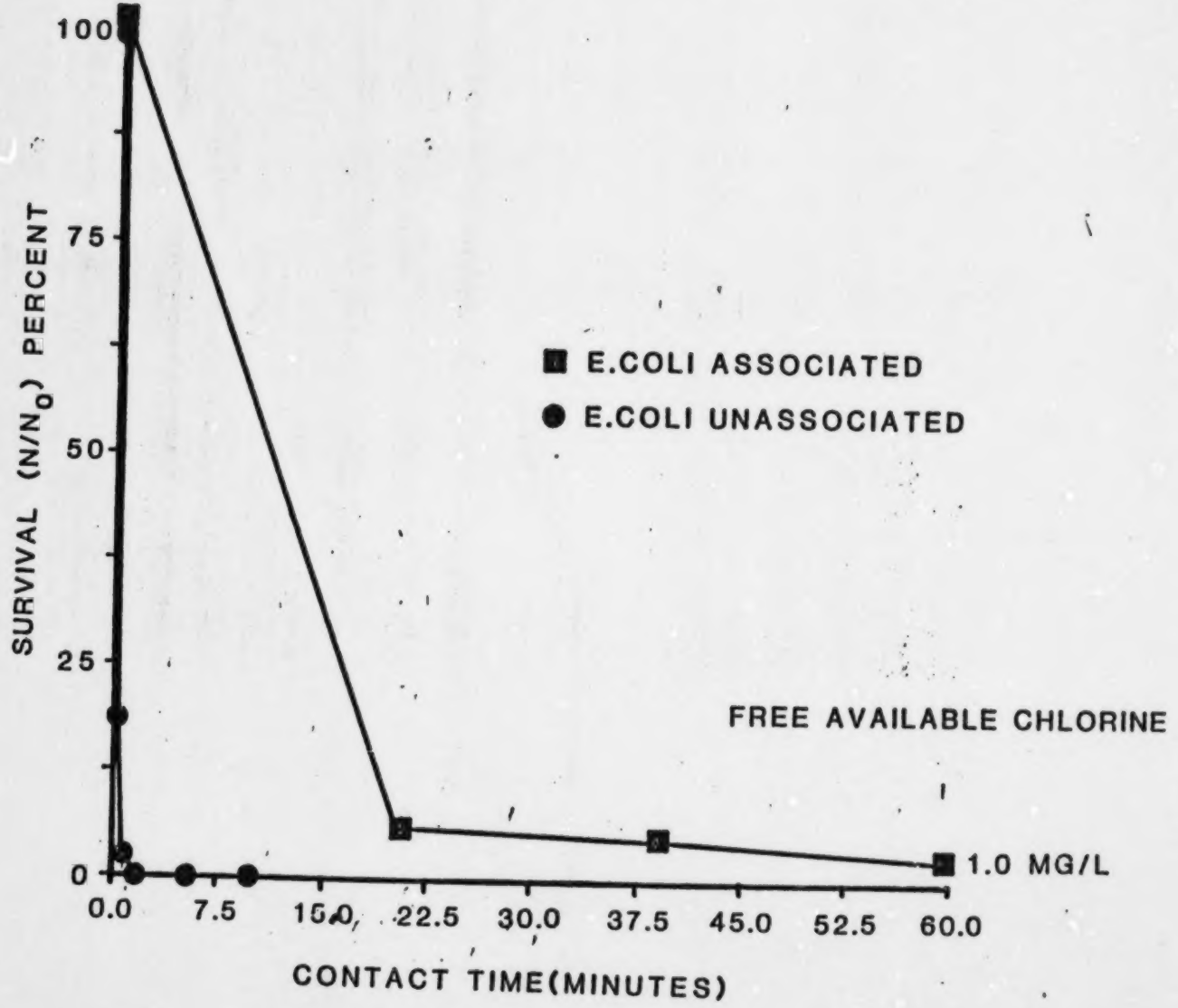

100
75
50
25
0
SURVIVAL (N/N0) PERCENT
E.COLI ASSOCIATED
E.COLI UNASSOCIATED
FREE AVAILABLE CHLORINE
1.0 MG/L
0.0
7.5
15.0
22.5
30.0
37.5
45.0
52.5
60.0
CONTACT TIME(MINUTES)

FIGURE 6

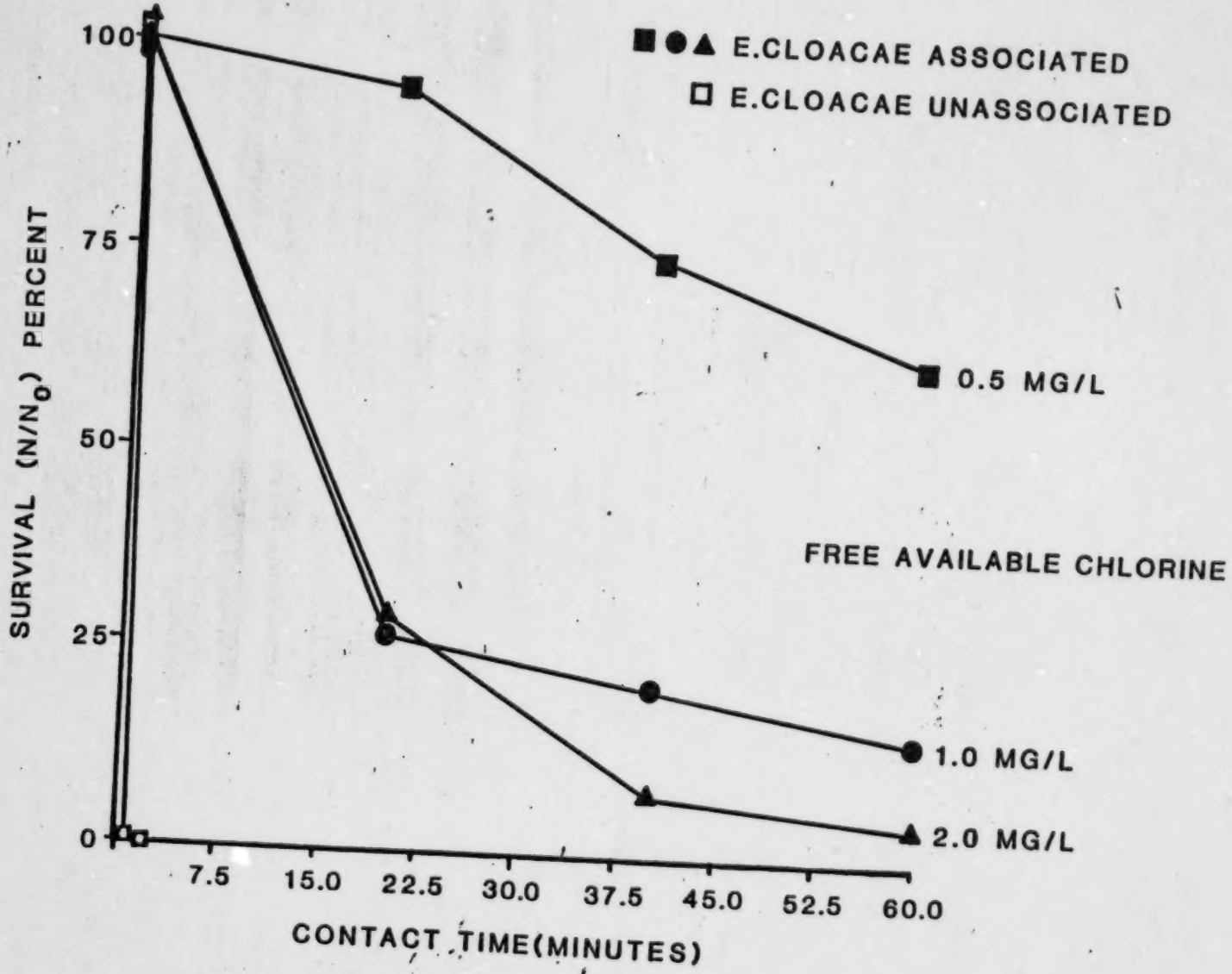

E.CLOACAE ASSOCIATED
E.CLOACAE UNASSOCIATED
SURVIVAL (N/N0) PERCENT
100
75
50
25
0
0.5 MG/L
FREE AVAILABLE CHLORINE
1.0 MG/L
2.0 MG/L
7.5
15.0
22.5
30.0
37.5
45.0
52.5
60.0
CONTACT TIME(MINUTES)

FIGURE 7

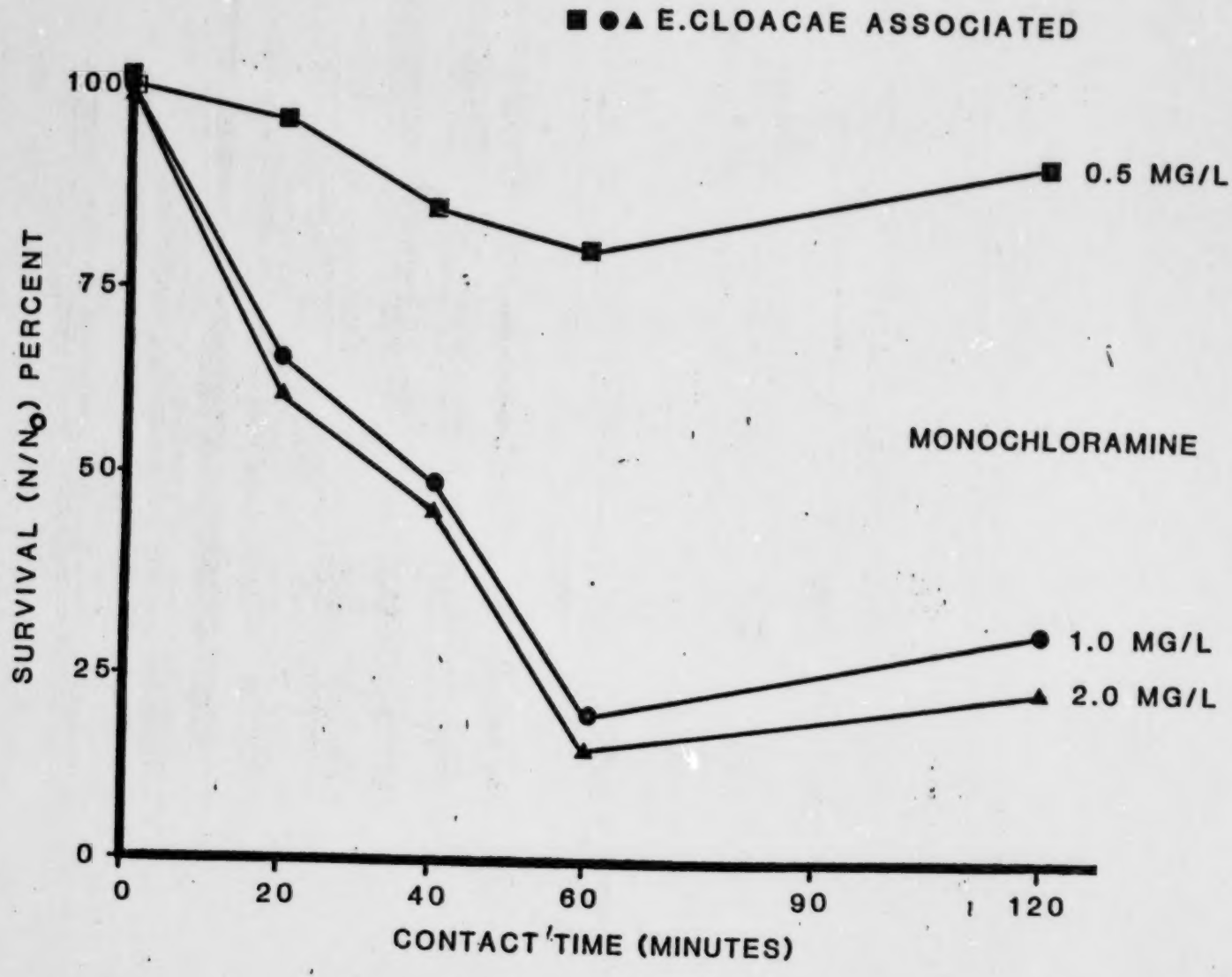

■ ● ▲ E.CLOACAE ASSOCIATED
100
75
50
25
0
SURVIVAL (N/N$_o$) PERCENT
0.5 MG/L
MONOCHLORAMINE
1.0 MG/L
2.0 MG/L
0
20
40
60
90
120
CONTACT TIME (MINUTES)

FIGURE 8